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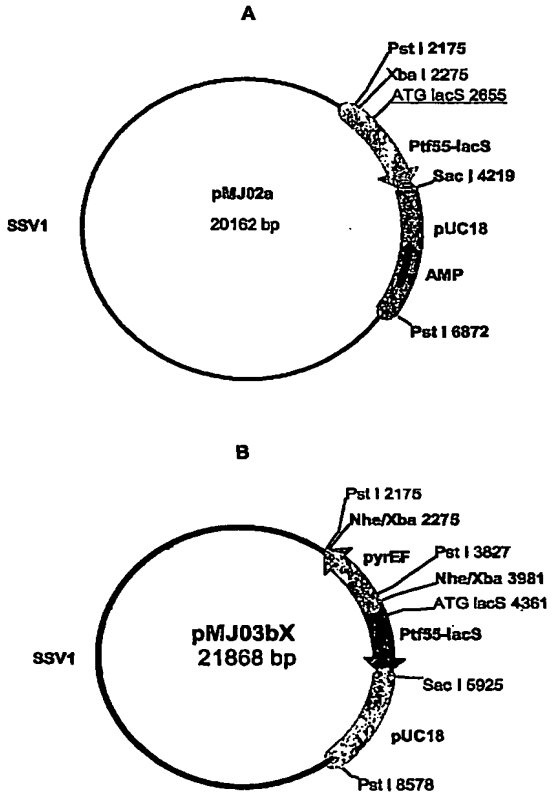
(54) Title: ARCHAEON EXPRESSION SYSTEM




(57) Abstract: The present invention relates to a sulfolobus expression vector comprising: (a) sulfolobus origin of replication; (b) the genes encoding the structural proteins and the site-specific integrase of SSVI, SSV2 or pSSVx, operatively linked to expression control sequences and a packaging signal; (c) one or more selectable marker gene(s), operatively linked to sulfolobus expression control sequences; and (d) a sulfolobus promoter followed 3' by a restriction enzyme recognition site or a multiple cloning site for insertion of a gene of interest and optionally a 3' regulatory element. Moreover, the present invention relates to a shuttle vector comprising the sequences of the expression vector of the invention and additional sequences for propagation and selection in E. coli, wherein the additional sequences comprise (a) an E.coli on of replication; and (b) a marker for selection in E.coli. Furthermore, the invention relates to host cells transformed with the expression vector as well as to a kit comprising a vector or a host cell of the present invention. Finally, the present application also relates to a method for generating infectious subviral particles.

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*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

**AMENDED CLAIMS**
[received by the International Bureau on 02 November 2004 (02.11.04);
original claims 1-21 are replaced by amended claims 1-20]

# CLAIMS

1. A sulfolobus expression vector comprising:
   (a) a sulfolobus origin of replication;
   (b) the genes encoding the structural proteins and the site-specific integrase of SSV1, SSV2 or pSSVx, operatively linked to expression control sequences and a packaging signal;
   (c) one or more selectable marker gene(s) encoding an essential protein of sulfolobus, operatively linked to sulfolobus expression control sequences; and
   (d) a sulfolobus promoter followed 3' by a restriction enzyme recognition site or a multiple cloning site for insertion of a gene of interest and optionally a 3' regulatory element.

2. The expression vector of claim 1, wherein the origin of replication of (a) is selected from the group consisting of SSV1, SSV2, pSSVx and pRN plasmids.

3. The expression vector of claim 1 or 2, wherein the vector contains the complete genome of SSV1, thereby providing said origin of replication, said packaging signal and said genes encoding the structural proteins and the integrase of SSV1.

4. The expression vector of claim 3, wherein the essential gene is a gene of the de novo nucleotide anabolism, a gene of the aminoacid biosynthesis or a gene conferring antibiotic resistance

5. The expression vector of anyone of claims 1 to 4, wherein the vector contains orotidine-5'-monophosphatase pyrophosphorlyase and orotidine-5'-monophosphatase decarboxylase as selectable marker genes.

6. The expression vector of any one of claims 1 to 5, wherein the vector contains 3' to the translation initiation site of the promoter for the expression of the gene of interest additional nucleic acid sequences so that the expressed protein has an N-terminal extension.

7. The expression vector of claim 6, wherein the N-terminal extension is
   (a) a signal sequence directing the secretion of the expressed protein;
   (b) a tag for purification; or
   (c) a tag for specific detection.

8. The expression vector of any one of claims 1 to 7, wherein the promoter for the expression of the gene of interest is a constitutive promoter selected from the group consisting of genes involved in central metabolisms and information processing including the promoters of the ribosomal subunits 16S, 23S rRNA or the promoters of polymerases, transcription, replication or translation factors.

9. The expression vector of any one of claims 1 to 8, wherein the promoter for the expression of the gene of interest is an inducible promoter.

10. The expression vector of claim 9, wherein the inducible promoter is selected from the group consisting of (a) heat inducible promoters Tf55alpha, TF55beta, TF55gamma, hsp20, htrA, (b) cold inducible promoters TF55gamma and (c) promoters inducible by a carbon source.

11. The expression vector of any one of claims 1 to 10, wherein the vector contains an additional expression cassette for a reporter protein, selected from the group consisting of ß-galactosidase, luciferase, green fluorescent protein and variants thereof.

12. A shuttle vector comprising the sequences of the expression vector of any one of claims 1 to 11 and additional sequences for propagation and selection in E. coli, wherein the additional sequences comprise
    (a) an E.coli ori of replication; and
    (b) a marker for selection in E.coli.

13. The shuttle vector of claim 12, wherein the marker of selection is selected from the group consisting of ampicillin, kanamycin, chloramphenicol, tetracyclin, hygromycin, neomycin or methotrexate.

14. A host cell transformed with the expression vector of any one of claims 1 to 13, wherein the host cell is E. coli or sulfolobus.

15. The host cell of claim 14, wherein the transformed expression vector provides a gene encoding an essential protein.

16. The host cell of claim 14, wherein the host is deficient in expressing a fully functional version of said essential gene provided by the expression vector.

17. A method of producing a polypeptide comprising culturing the host cell of any one of claims 14 to 17 under suitable conditions and isolating said (poly)peptide from the cells or the cell culture supernatant.

18. A method of generating infectious recombinant subviral particles composed of the structural proteins of SSV1 and/or SSV2, having packaged the DNA of the expression vector of any one of claims 1 to 13, wherein the method has the steps of
    (a) introducing the DNA of the expression vector and the DNA of SSV1 or SSV2 into a host cells;
    (b) incubating the cells for time and under conditions sufficient to allow replication of SSV1 or SSV2 and spreading in the cell culture;
    (c) harvesting the cell culture supernatant or the host cells.

19. Use of the vector of any one of claims 1 to 13 for gene silencing by expression of RNAi or antisense RNA, wherein the vector contains a Sulfolobus promoter for transcription of a gene or parts of a gene either in antisense or sense orientation or in both orientations.

20. A kit comprising
    (a) the vector of any one of claims 1 to 13,
    (b) the host cell of any one of claim 14 to 16, and/or
    (c) a host cell deficient in the expression of the essential protein of the vector of (a).

    in one or more containers.